

Figure 1. Nucleotide and amino acid sequences (SEQ ID Nos: 1 and 2) of the *C. pneumoniae* 60 kDa cysteine rich membrane protein

ttgatcagg t gtttggaga tgaatttaatt cctgactacc ttaattcaga taataaaccc	60
aaatgttgag ggtaagagtt tacaaaacat tctacccgat ggcagaagaa aaaaataaac	120
atgcgatagg agatccct atg tcc aaa ctc atc aga cga gta gtt acg gtc	171
Met Ser Lys Leu Ile Arg Arg Val Val Thr Val	
1 5 10	
ctt gcg cta acg agt atg gcg agt tgc ttt gcc agc ggg ggt ata gag	219
Leu Ala Leu Thr Ser Met Ala Ser Cys Phe Ala Ser Gly Gly Ile Glu	
15 20 25	
gcc gct gta gca gag tct ctg att act aag atc gtc gct agt gcg gaa	267
Ala Ala Val Ala Glu Ser Leu Ile Thr Lys Ile Val Ala Ser Ala Glu	
30 35 40	
aca aag cca gca cct gtt cct atg aca gcg aag aag gtt aga ctt gtc	315
Thr Lys Pro Ala Pro Val Pro Met Thr Ala Lys Lys Val Arg Leu Val	
45 50 55	
cgt aga aat aaa caa cca gtt gaa caa aaa agc cgt ggt gct ttt tgt	363
Arg Arg Asn Lys Gln Pro Val Glu Gln Lys Ser Arg Gly Ala Phe Cys	
60 65 70 75	
gat aaa gaa ttt tat ccc tgt gaa gag gga cga tgt caa cct gta gag	411
Asp Lys Glu Phe Tyr Pro Cys Glu Gly Arg Cys Gln Pro Val Glu	
80 85 90	
gct cag caa gag tct tgc tac gga aga ttg tat tct gta aaa gta aac	459
Ala Gln Gln Glu Ser Cys Tyr Gly Arg Leu Tyr Ser Val Lys Val Asn	
95 100 105	
gat gat tgc aac gta gaa att tgc cag tcc gtt cca gaa tac gct act	507
Asp Asp Cys Asn Val Glu Ile Cys Gln Ser Val Pro Glu Tyr Ala Thr	
110 115 120	
gta gga tct cct tac cct att gaa atc ctt gct ata ggc aaa aaa gat	555
Val Gly Ser Pro Tyr Pro Ile Glu Ile Leu Ala Ile Gly Lys Lys Asp	
125 130 135	
tgt gtt gat gtt gtg att aca caa cag cta cct tgc gaa gct gaa ttc	603
Cys Val Asp Val Val Ile Thr Gln Gln Leu Pro Cys Glu Ala Glu Phe	
140 145 150 155	
gta agc agt gat cca gaa aca act cct aca agt gat ggg aaa tta gtc	651
Val Ser Ser Asp Pro Glu Thr Thr Pro Thr Ser Asp Gly Lys Leu Val	
160 165 170	
tgg aaa atc gat cgc ctg ggt gca gga gat aaa tgc aaa att act gta	699
Trp Lys Ile Asp Arg Leu Gly Ala Gly Asp Lys Cys Lys Ile Thr Val	
175 180 185	

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Figure 1 (cont'd)

tgg gta aaa cct ctt aaa gaa ggt tgc tgc ttc aca gct gct act gta 747  
 Trp Val Lys Pro Leu Lys Glu Gly Cys Cys Phe Thr Ala Ala Thr Val  
 190 195 200

tgt gct tgc cca gag ctc cgt tct tat act aaa tgc ggt caa cca gcc 795  
 Cys Ala Cys Pro Glu Leu Arg Ser Tyr Thr Lys Cys Gly Gln Pro Ala  
 205 210 215

att tgt att aag caa gaa gga cct gac tgt gct tgc cta aga tgc cct 843  
 Ile Cys Ile Lys Gln Glu Gly Pro Asp Cys Ala Cys Leu Arg Cys Pro  
 220 225 230 235

gta tgc tac aaa atc gaa gta gtg aac aca gga tct gct att gcc cgt 891  
 Val Cys Tyr Lys Ile Glu Val Val Asn Thr Gly Ser Ala Ile Ala Arg  
 240 245 250

aac gta act gta gat aat cct gtt ccc gat ggc tat tct cat gca tct 939  
 Asn Val Thr Val Asp Asn Pro Val Pro Asp Gly Tyr Ser His Ala Ser  
 255 260 265

ggt caa aga gtt ctc tct ttt aac tta gga gac atg aga cct ggc gat 987  
 Gly Gln Arg Val Leu Ser Phe Asn Leu Gly Asp Met Arg Pro Gly Asp  
 270 275 280

aaa aag gta ttt aca gtt gag ttc tgc cct caa aga aga ggt caa atc 1035  
 Lys Lys Val Phe Thr Val Glu Phe Cys Pro Gln Arg Arg Gly Gln Ile  
 285 290 295

act aac gtt gct act gta act tac tgc ggt gga cac aaa tgt tct gca 1083  
 Thr Asn Val Ala Thr Val Thr Cys Gly Gly His Lys Cys Ser Ala  
 300 305 310 315

aat gta act aca gtt gtt aat gag cct tgt gta caa gta aat atc tct 1131  
 Asn Val Thr Thr Val Val Asn Glu Pro Cys Val Gln Val Asn Ile Ser  
 320 325 330

ggt gct gat tgg tct tac gta tgt aaa cct gtg gag tac tct atc tca 1179  
 Gly Ala Asp Trp Ser Tyr Val Cys Lys Pro Val Glu Tyr Ser Ile Ser  
 335 340 345

gta tcg aat cct gga gac ttg gtt ctt cat gat gtc gtg atc caa gat 1227  
 Val Ser Asn Pro Gly Asp Leu Val Leu His Asp Val Val Ile Gln Asp  
 350 355 360

aca ctc cct tct ggt gtt aca gta ctc gaa gct cct ggt gga gag atc 1275  
 Thr Leu Pro Ser Gly Val Thr Val Leu Glu Ala Pro Gly Gly Glu Ile  
 365 370 375

tgc tgt aat aaa gtt gtt tgg cgt att aaa gaa atg tgc cca gga gaa 1323  
 Cys Cys Asn Lys Val Val Trp Arg Ile Lys Glu Met Cys Pro Gly Glu  
 380 385 390 395

acc ctc cag ttt aaa ctt gta gtg aaa gct caa gtt cct gga aga ttc 1371  
 Thr Leu Gln Phe Lys Leu Val Val Lys Ala Gln Val Pro Gly Arg Phe  
 400 405 410

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aca aat caa gtt gca gta act agt gag tct aac tgc gga aca tgt aca 1419  
 Thr Asn Gln Val Ala Val Thr Ser Glu Ser Asn Cys Gly Thr Cys Thr  
 415 420 425

Figure 1 (cont'd)

tct tgc gca gaa aca aca aca cat tgg aaa ggt ctt gca gct acc cat 1467  
 Ser Cys Ala Glu Thr Thr His Trp Lys Gly Leu Ala Ala Thr His  
 430 435 440  
 atg tgc gta tta gac aca aat gat cct atc tgt gta gga gaa aat act 1515  
 Met Cys Val Leu Asp Thr Asn Asp Pro Ile Cys Val Gly Glu Asn Thr  
 445 450 455  
 gtc tat cgt atc tgt gta act aac cgt ggt tct gct gaa gat act aac 1563  
 Val Tyr Arg Ile Cys Val Thr Asn Arg Gly Ser Ala Glu Asp Thr Asn  
 460 465 470 475  
 gta tct tta atc ttg aag ttc tca aaa gaa ctt cag cca ata gct tct 1611  
 Val Ser Leu Ile Leu Lys Phe Ser Lys Glu Leu Gln Pro Ile Ala Ser  
 480 485 490  
 tca ggt cca act aaa gga acg att tca ggt aat acc gtt gtt ttc gac 1659  
 Ser Gly Pro Thr Lys Gly Thr Ile Ser Gly Asn Thr Val Val Phe Asp  
 495 500 505  
 gct tta cct aaa ctc ggt tct aag gaa tct gta gag ttt tct gtt acc 1707  
 Ala Leu Pro Lys Leu Gly Ser Lys Glu Ser Val Glu Phe Ser Val Thr  
 510 515 520  
 ttg aaa ggt att gct ccc gga gat gct cgc ggc gaa gct att ctt tct 1755  
 Leu Lys Gly Ile Ala Pro Gly Asp Ala Arg Gly Glu Ala Ile Leu Ser  
 525 530 535  
 tct gat aca ctg act tca cca gta tca gac aca gaa aat acc cac gtg 1803  
 Ser Asp Thr Leu Thr Ser Pro Val Ser Asp Thr Glu Asn Thr His Val  
 540 545 550  
 tat taa attctaagga attatcctaa agcagagcga tattccgctc tgcttttagga 1859  
 Tyr  
 tagcttcaa agaagtaccg cttagtacc ttacgtacta aagcggtttt tttgttttat 1919  
 aagctcttca atccaatcgt agagttctt aatcaaagat attatthaag tttctgaaat 1979  
 cctaaagattt attttaaaag cccatcttt taggtatgta attaaaattt ttaattaagc 2039  
 ttttccttagt gtaacctgct tcttttaggaa ctacactagg agaacggat gtcataaat 2099  
 2111  
 ctacatccccg ta

Figure 2: Restriction enzyme analysis of the the *C. pneumoniae* 60 kDa cysteine rich membrane protein.

	1	528	1056	1583	2111
enzyme	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
Acc113I			! !		
Acc16I				!	
AccBSI					!
AccII					!
AciI	! ! !		! ! !	! ! !	! ! !
AclI			!		
AclNI				!	
AclWI	!	! ! !	!	! ! !	!
AcsI	!	! ! !			! ! !
AfaI			! ! !	! ! !	*
AflIII				!	
AluI		*	! !	! ! !	! ! !
Alw21I			!		
Alw26I	!		*	!	
AlwI	!	! ! !	!	! ! !	!
AlwNI	!		!		
ApoI		! ! !			! ! !
AseI	!				
AsnI	!				
Asp700I	!			! !	
AspHI			!		
AspI		!			
AspLEI		!		!	
AspS9I			!		!
AsuC2I				!	
AsuHPI				!	
AvaII	!		!		!
AvI				!	
BanII			!		
BanIII			!		
BbrPI					!
Bbv12I			!		
BbvI		*		!	
BclI	!				
BcnI					!
BfaI		!		!	!
BfmI		! ! !	!	!	!
BglII				!	
BlpI	!				
Bme18I	!		!		!
BmyI			!		!
BpmI				!	!
Bpu1102I		!			
Bsa29I		!			
BsaAI			!		
BsaI			!		
BsaJI	!	!		!	!
BsaOI		!			
Bsc4I				!	!
BscI		!			

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Figure 2 (cont'd)

BseiI	! ! !
BsecI	! !
BseDI	! ! !
BseNI	! ! !
BsgI	!
Bsh1236I	! !
Bsh1285I	!
Bsiei	!
BsiHKAI	!
Bsili	! ! ! ! !
Bsiqi	!
Bsisi	! !
Bsiyi	! !
Bsizi	! !
Bsli	! !
BsmAI	! * !
BsmFI	!
Bsp106I	! !
Bsp1286I	! !
Bsp1407I	! !
Bsp1720I	!
BspHI	!
Bsplu11I	!
BspMI	!
BspXI	!
BsrBI	!
BsrgI	! !
Bsri	! !
BsrsI	! !
Bst2UI	! ! ! ! !
Bst7II	* ! ! ! !
BstBAI	! ! ! ! !
BstDEI	! ! ! ! !
BstdSI	! !
BstmCI	!
BstnSI	!
BstoI	! ! ! ! !
BstsFI	! ! ! ! !
BstsNI	! !
BstuI	!
Bstx2I	! ! ! ! !
BstyI	! ! ! ! !
Bsu15I	!
Bsu6I	! ! ! ! !
Cac8I	! ! ! ! !
CeliI	!
Cfr13I	! ! !
Clai	!
Cviji	* ! ! ! ! ! ! ! ! ! ! ! ! !
DdeI	! ! ! ! !
Dpni	! ! ! ! !
Drai	!
DraiI	!
Dsai	!
Eam1104I	! ! !
Eari	!
Eco105I	!
Eco24I	! !

Figure 2 (cont'd)

Eco31I	! ! !
Eco47I	! ! ! * !
Eco57I	! ! !
Eco72I	! ! !
EcorI	! ! !
EcorII	! ! ! ! ! ! !
Ecot22I	! ! !
Ecot38I	! ! !
FauI	! !
FbaI	! !
Fnu4HI	! ! * ! ! !
FokI	! ! !
FriOI	! !
Fsp4HI	! ! * ! ! !
FspI	! ! !
GsuI	! ! !
HaeIII	! ! !
HapII	! ! !
Hgai	! ! !
HgiEI	! ! !
Hhai	! ! !
Hin6I	! ! !
HindII	! ! !
HindIII	! ! !
HinfI	! ! ! ! ! ! !
HpaII	! ! !
Hphi	! ! !
Hsp92II	! ! ! ! ! ! !
HspAI	! ! !
Itai	! ! * ! ! !
Ksp22I	! ! !
Ksp632I	! ! !
Kzo9I	! ! ! ! ! ! !
MaeI	! ! !
MaeII	! ! !
MaeIII	! ! * ! ! ! ! !
MboI	! ! ! ! ! ! !
MboII	! ! ! ! ! ! !
Mfli	! ! ! ! ! ! !
Mnli	! ! * ! ! * ! !
Mphi103I	! !
MroXI	! ! ! ! ! ! !
MseI	! ! ! ! ! ! ! ! ! ! ! ! !
MspAII	! ! !
MspR9I	! ! ! ! ! ! !
Mvni	! ! !
MwoI	! ! * !
NciI	! ! !
NdeI	! ! !
NlaIII	! ! ! ! ! !
NsiI	! ! !
NspBII	! ! !
NspI	! ! !
PacI	! ! !
PalI	! ! !
Ple19I	! ! !
PlefI	! ! !
PmaCI	! ! !

Figure 2 (cont'd)

PmeI	
PmlI	
Ppu10I	
PpuMI	
PshBI	
Psp124BI	
Psp1406I	
Psp5II	
PspPPI	
PvuI	
PvuII	
RcaI	
RsaI	
SacI	
SapI	
Sau96I	
ScaI	
ScrFI	
SduI	
SfaNI	
SfcI	
SnaBI	
SpeI	
Sse9I	* ! _____ ! _____ ! ! ! * * *
SspBI	
TaiI	
TaqI	
TfiI	
ThaI	
Tru11I	! ! _____ ! ! ! ! ! ! ! ! ! ! ! ! ! ! * !
Tru9I	! ! _____ ! ! ! ! ! ! ! ! ! ! ! ! ! ! ! ! * !
TscI	
TseI	*
Tsp509I	* ! _____ ! ! ! ! ! ! ! ! ! ! ! ! ! ! ! ! * * *
TspEI	* ! _____ ! ! ! ! ! ! ! ! ! ! ! ! ! ! ! ! * * *
TspRI	
Tth111I	
VspI	
XbaII	
XmnI	
Zsp2I	

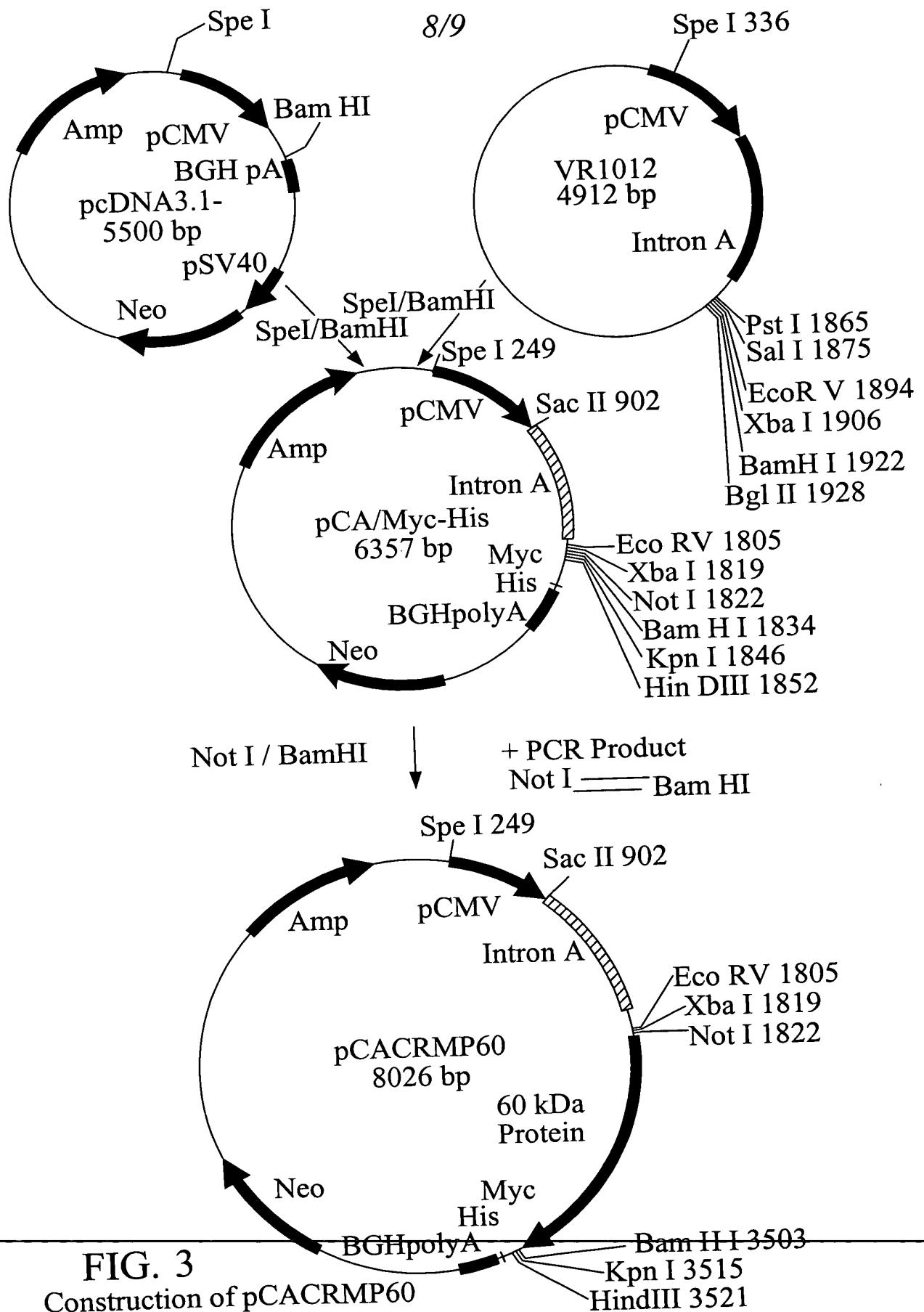


FIG. 3  
Construction of pCACRMP60

Figure 4: Protective efficacy of DNA immunization with pCACRMP60 against intranasal challenge of *C. pneumoniae*

